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# THE OPTIMUM HYDROGEN-ION CONCENTRATION FOR THE GROWTH OF *B. TYPHOSUS*, AND *B.* *PARATYPHOSUS A* AND *B*

EXPERIMENTAL TYPHOID-PARATYPHOID CARRIERS. II

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It is known that *B. typhosus* and the allied organisms of this group grow best in a neutral or slightly acid medium. The present study was undertaken to determine the probable range of growth and the optimum H-ion concentration.

There are two methods commonly employed to measure the H-ion concentration of a solution. The first is by means of the H-electrode, the second is the colorimetric method. For very accurate work, in which slight changes in H-ion must be determined, or in which the greatest possible accuracy is to be attained, the first method of measuring H-ion concentration is generally used. However, when the changes in H-ion concentration extend over a broad range, and when the method of experiment calls for a large number of determinations of only relative accuracy, the second method is to be preferred. This is particularly true when the solutions to be tested are more or less complex and the salt and protein content interferes with the electrometric determinations.

The ability of certain solutions to resist changes in H-ion concentration is called its buffer action. The body fluids, ordinary Witte's peptone solution, solutions of weak acids, such as phosphates, borates and acetates, and many others, exhibit this property. This behavior of certain substances in solution has been used by several investigators in the preparation of solutions of known H-ion concentration. Sørensen<sup>1</sup> carefully studied such a set, as did Walpole<sup>2</sup> and Palitsch<sup>3</sup>; Clark and Lubs<sup>4</sup> have recently modified these sets slightly. In our work, we have found it necessary to prepare these buffer solutions in a range extend-

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<sup>1</sup> Biochem. Ztschr., 1907, 7, p. 131.

<sup>2</sup> Biochem. Jour., 1910, 5, p. 207.

<sup>3</sup> Biochem. Ztschr., 1912, 47, p. 1.

<sup>4</sup> Jour. Bacteriol., 1917, 2, p. 1.

ing from  $P_H + 4.0$  to  $P_H + 9.0$ . These solutions were carefully prepared<sup>5</sup> and checked (through the kindness of Dr. Schmidt) with a H-electrode.

Up to recent years one of the main objections to the use of the colorimetric method for the determination of H-ion concentration in bacteriologic work was the scarcity of brilliant indicators covering the entire range of H-ion concentration. Phenolphthalein, an indicator most commonly used, has a range on the alkaline side from  $P_H + 8.0$  to  $P_H + 10.0$ . Litmus is not sensitive or brilliant enough for colorimetric work. Methyl red has been used and has proved very satisfactory. It passes from its full acid color into its alkaline color as the H-ion concentration falls from  $P_H + 4.8$  to  $P_H + 6.4$ . Through the work of Clark and Lubs,<sup>4</sup> however, several new and brilliant indicators have become available for this work. Methyl red, bromthymol blue, cresol red and thymol blue were the indicators most generally used in this work.

There has been a great deal of discussion relative to the death point and its relation to the H + ion concentration. Bruenn,<sup>6</sup> using mixtures of lactates as well as acetates of a definite H + ion concentration, came to the conclusion that the disinfection which takes place at high H + ion concentrations depends on the  $P_H +$ . He found that the death point of *B. coli* was between  $P_H 4.7$  and  $4.4$ ; for *B. typhosus* between  $P_H + 5.6$   $5.0$ - $4.7$ . We have not noted quite as large a range in our experiments, as will be indicated later. Winslow and Lockridge<sup>7</sup> have shown that typhoid bacilli are a little less than half as resistant as *B. coli* to dilute acids. Using HCl and  $H_2SO_4$ , the toxicity depends on the H + ion concentration; the undissociated part of the molecule exerts no toxic effect. Organic acids, such as benzoic or acetic acids, are toxic for these organisms in strengths in which they are only slightly dissociated. The toxicity is the result of the undissociated part of the molecule. The accumulation of similar organic acids might account for the phenomena observed by Clark, that the final H + ion concentration is lower in more highly buffered mediums.

In order to determine the degree of acidity or alkalinity necessary to inhibit *B. coli* in the animal body, Shohl and Janney<sup>8</sup> studied the growth of these organisms in urine at varying H + ion concentrations. They

<sup>5</sup> We are indebted to Mr. P. Edson for the preparation and standardization of these solutions.

<sup>6</sup> Jour. Urology, 1917, 1, p. 211.

<sup>7</sup> Jour. Infect. Dis., 1906, 3, p. 547.

<sup>8</sup> Jour. Urology, 1917, 1, p. 211.

showed that these organisms were inhibited in their growth at a  $P_H + 0$  of 4.6-5.0 on the acid side, and from  $P_H + 9.2$ -9.6 on the alkaline side. The optimum growth for *B. coli* was between 6.0-7.0. They also tested the Rawlings strain of *B. typhosus* (our strain 6) and state that the typhoid bacillus has narrower limits, showing no growth at  $P_H + 5.0$  on the acid side, or  $P_H + 8.8$  on the alkaline side. Quite recently Winslow, Kligler and Rothberg<sup>9</sup> found that *B. typhosus* (Rawlings' strain) produces, inside of 24-96 hours, in hexoses an acid reaction of  $P_H + 5.5$ -5.0, which remains practically constant.

TABLE 1

HYDROGEN-ION CONCENTRATION OF MIXTURES OF SALT-FREE BROTH, HYDROCHLORIC ACID OR SODIUM HYDROXIDE AND PHOSPHATES

10 cc broth + 2 cc 5M phosphates + HCl or NaOH + H<sub>2</sub>O = 13 cc

Tube No.	0.1 n NaOH, C c	0.1 n HCl, C c	0.5 n KH <sub>2</sub> PO <sub>4</sub> , C c	0.5 n Na <sub>2</sub> HPO <sub>4</sub> , C c	Hydrogen-ion Concentration of Mixture, $P_H$
		$P_H$ of Medium = 7.4			
	3.0	...	...	...	9.0
1	2.4	...	...	2	8.8
2	2.4	...	...	2	8.6
3	2.0	...	0.04	1.96	8.4
4	1.3	...	0.1	1.9	8.2
5	1.0	...	0.12	1.88	8.0
6	0.6	...	0.14	1.86	7.8
7	...	...	0.2	1.8	7.6
8	...	...	0.4	1.6	7.4
9	...	...	0.7	1.3	7.2
10	...	0.5	0.8	1.2	7.0
11	...	0.6	1.0	1.0	6.8
12	...	0.8	1.2	0.8	6.6
13	...	1.1	1.45	0.55	6.4
14	...	1.6	1.7	0.3	6.0
		$P_H$ of Medium = 6.4			
15	...	0.6	1.7	0.3	6.0
16	...	0.8	1.8	0.2	5.8
17	...	1.0	1.9	0.1	5.4
18	...	1.7	2	...	5.0
19	...	2.1	2	...	4.8
20	...	2.4	...	...	4.3

The exact effect of variations in the  $H +$  ion concentration of culture mediums on *B. typhosus* has not been investigated extensively. It was thought advisable to investigate this problem before attempting other work in which variations in the  $H +$  ion concentration are concerned. Moreover, it will be evident from the observations reported in subsequent papers (6, 7 and 8) of this series, that a detailed knowledge of the  $H$ -ion requirements of an organism is absolutely essential before an explanation can be attempted relative to the behavior of a bacterium in the body fluids or the secretions of an animal.

<sup>9</sup> Jour. Bacteriol., 1919, 4, p. 471.

*Technic.*—Preparation of Mediums: The mediums were prepared by allowing 500 gm. of lean veal to infuse in 1000 cc of tap water on ice over night. This infusion was boiled for 20 minutes, strained through cheese cloth and allowed to cool. The fat was removed by filtration, 1% "Difco" peptone was added, and the medium adjusted to a reaction of  $P_H + 7.4$ . After stabilization in the Arnold for 30 minutes, the broth was filtered and sterilized by fractional sterilization in live steam for 3 consecutive days. This "salt free medium" was made in large quantities. The same lot of medium was used for the entire series of experiments.

TABLE 2  
INFLUENCE OF SALT CONCENTRATION ON THE GROWTH OF *B. TYPHOSUS*  
INCUBATION 18 HOURS AT 37 C.

P <sub>H</sub> 7.0 Molal Concen- tration	Growth			
	NaCl		KCl	
	Kearney	Blair	Kearney	Blair
1.0	—	—	—	—
0.5	++	++	++	++
0.3	+++	+++	+++	+++
0.1	+++	+++	+++	+++
0.06	+++	+++	+++	+++
0.04	+++	+++	+++	+++
0.02	+++	+++	+++	+++
Salt free broth	+++	+++	+++	+++

TABLE 3  
INFLUENCE OF PHOSPHATES ON *B. TYPHOSUS* BROTH AND PHOSPHATE MIXTURE  
(7 PARTS  $Na_2HPO_4$  + 3 PARTS  $KH_2PO_4$ )

+ 0.1 cc of 1:10,000 20-hour broth culture + distilled  $H_2O$  = 5 cc

Strain								
Kearney			Jacobs			Blair		
Tube Number	Molal Concentration of Phosphate	Growth	Tube Number	Molal Concentration	Growth	Tube Number	Molal Concentration	Growth
20	0.4	—	25	0.4	—	30	0.4	—
21	0.3	++	26	0.3	++	31	0.3	++
22	0.2	+++	27	0.2	+++	32	0.2	+++
23	0.1	+++	28	0.1	+++	33	0.1	+++
24	...	+++	29	...	+++	34	...	+++
Salt-free broth			Salt-free broth			Salt-free broth		

To each 10 cc of medium NaOH or HCl was added in order to obtain the desired reaction. Two cc of 0.5M  $Na_2HPO_4$  and 0.5M  $KH_2PO_4$  mixtures ( $Na_2HPO_4$  being alkaline and  $KH_2PO_4$  being acid in reaction) were added in order to keep the H-ion concentration constant during the course of the experiment. By combining these solutions in certain proportions a series of tubes varying in reaction from  $P_H + 4.3$  to  $P_H + 9.0$  were obtained. Such a series is shown in table 1.

Three c.c quantities were tubed and inoculated with 0.1 c.c of a 1:10,000 dilution of a 24-hour "salt-free broth" culture. Growth was determined after 24 hours' incubation by the plate method. Triplicate plates were poured with veal infusion agar,  $P_H + 6.8-7.2$ , and counted after 24 hours. The remainder of the culture was sterilized in the Arnold for 30 minutes and the H-ion concentration determined. A control tube was also incubated and heated in the same way and the H-ion concentration determined. Variations in the H-ion concentrations were seldom found, except on the extreme alkaline side. These results are in accord with those obtained by Dernby and Avery<sup>10</sup>, in their work on the growth of pneumococcus.

*Isolation of Strains.*—The strains were isolated on brilliant green mediums and transferred to peptic digest agar slants. For the experiments the cultures were purified on brilliant green or plain agar plates. Single colonies were transferred to veal infusion agar slants ( $P_H + 6.8-7.2$ ) and kept at room temperature.

TABLE 4  
OPTIMUM GROWTH OF *B. TYPHOSUS*; STOCK CULTURES

Blair 2*			Jacobs†			Number 1‡			Kearney§		
$P_H$ Cont.	$P_H$ 24- Hour Cul- ture	Growth per C c	$P_H$ Cont.	$P_H$ 24- Hour Cul- ture	Growth per C c	$P_H$ Cont.	$P_H$ 24- Hour Cul- ture	Growth per C c	$P_H$ Cont.	$P_H$ 24- Hour Cul- ture	Growth per C c
4.3	4.3	2,000				4.6	4.5	2,000	4.3	4.3	
4.8	4.8	97,000	4.8	4.8	0	4.9	4.8	2,000	4.8	4.8	<10,000
5.0	5.0	41,000,000	5.1	5.1	21,600,000	6.6	6.4	423,000,000	5.2	5.2	92,500,000
6.4	6.4	480,000,000	6.6	6.4	383,000,000	6.6	6.6	645,000,000	5.4	5.4	185,000,000
6.6	6.6	580,000,000	6.8	6.6	508,000,000	6.8	6.8	606,000,000	5.6	5.6	225,000,000
6.8	6.8	876,000,000	7.0	6.8	480,000,000	7.0	7.0	650,000,000	6.0	6.0	960,000,000
7.0	7.0	400,000,000	7.4	7.0	260,000,000	7.2	7.2	525,000,000	6.6	6.4	1,500,000,000
7.4	7.4	236,000,000	8.3	7.9	216,000,000	7.4	7.4	482,000,000	6.8	6.8	1,500,000,000
8.4	8.4	143,000,000	8.9	8.9	<1,000,000	8.3	8.6	320,000,000	7.0	7.0	2,500,000,000
9.0	8.8	8,250				8.9	8.9	200,000	7.2	7.4	1,810,000,000
									7.6	7.4	1,400,000,000
									8.2	8.0	171,000,000
									8.4	8.3	3,000,000
									8.6	8.6	<100,000

\* Isolated from the blood in a severe case of typhoid complicated with cholecystitis and gall stones.

† Isolated from blood of a fatal case of typhoid, November, 1917.

‡ Isolated from a renal carrier, April, 1917.

§ Typical typhoid isolated from blood of a moderately severe case of typhoid, July, 1916.

*Experimental Data.*—The Influence of Salts on the Growth of *B. typhosus*: Preliminary experiments were conducted to determine the effect of varying concentrations of KCl, NaCl,  $KH_2PO_4$  and  $Na_2HPO_4$  on *B. typhosus*. The results of these experiments are shown in tables 2 and 3.

According to table 3 a 0.4 M solution of  $Na_2HPO_4$ - $KH_2PO_4$  mixture, is bacteriostatic, although it does prevent growth; a 0.3 M solution of phosphate is, however, more inhibitive than a 0.3 M solution of NaCl or KCl, etc. Since  $Na_2HPO_4$  and  $KH_2PO_4$  dissociate into 3 ions as compared to the 2 ions of the NaCl or KCl solution, a molal solution

of  $\text{Na}_2\text{HPO}_4$  or  $\text{KH}_2\text{PO}_4$  has a greater osmotic pressure than a molal solution of  $\text{KCl}$  or  $\text{NaCl}$ . The ratio of the comparative strength of the solution to the osmotic pressure is about as follows: Molal concentration of  $\text{NaCl}$ : molal concentration of  $\text{Na}_2\text{HPO}_4 = 2:3$  Isosmotic solutions of  $\text{NaCl}$  and  $\text{Na}_2\text{HPO}_4$ , therefore, produce about the same effect on *B. typhosus*. The phosphate concentration used in these experiments was kept far below that concentration which causes inhibition.

OPTIMUM GROWTH OF *B. TYPHOSUS* AND *B. PARATYPHOSUS* A. AND B.  
IN THE STANDARD "BUFFERED" MEDIUM

The determination of the optimum H-ion concentration for the growth of *B. typhosus* was conducted as previously stated. The results are shown in the following tables:

TABLE 5  
OPTIMUM GROWTH OF *B. TYPHOSUS*; STOCK CULTURES

Strain No. 3*			Strain No. 5†			Strain No. 7‡		
P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul- ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul- ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul- ture	Growth per C c
4.6	4.6	130	4.6	4.6	100	4.6	4.6	100
5.0	5.0	99,000	4.9	4.9	19,000	5.0	5.0	18,700
6.0	6.0	384,000,000	6.0	5.9	323,000,000	6.6	6.5	836,000,000
6.5	6.4	1,163,000,000	6.4	6.3	583,000,000	6.8	6.8	1,606,000,000
6.6	6.4	1,473,000,000	6.6	6.6	600,000,000	7.0	7.0	1,326,000,000
6.8	6.7	1,540,000,000	6.8	6.8	773,000,000	7.4	7.4	810,000,000
7.0	7.0	2,130,000,000	7.0	7.0	630,000,000	8.7	8.7	50,500,000
7.4	7.4	810,000,000	7.4	7.4	570,000,000			
7.8	7.8	530,000,000	8.6	8.1	353,000,000			
8.7	8.7	50,500,000						

\* Isolated from urine of patient suffering from renal calculi, November, 1916.

† Isolated from blood on the twelfth day of the disease, in a moderately severe case of typhoid, June, 1916.

‡ Isolated from blood in a moderately severe case of typhoid, October, 1916.

It will be seen from tables 4 to 7, inclusive, that the range of growth of *B. typhosus* is large. The optimum growth is between  $\text{P}_\text{H} + 6.8$ -7.0, or slightly on the acid side. Slight variations may occur, but most of the strains tested showed the same optimum range of growth. *B. coli* (Shohl and Janney), as compared to *B. typhosus*, shows a more pronounced optimum zone of growth and a large range. The results of 17 experiments on stock cultures have been averaged and are shown in the chart by the unbroken line. The logarithms of the number of organisms per cubic centimeter of medium are plotted as ordinates against the H-ion concentrations as abscissae.

TABLE 6  
OPTIMUM GROWTH OF B. TYPHOSUS; RECENTLY ISOLATED CULTURES

Eldridge*			Eldridge Strain†			Lung Strain‡			M. Strains§			Joe Strain¶			"C" Strain		
P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c
5.8	5.8	150,000,000	6.0	6.0	300,000,000	5.8	5.8	500,000,000	5.9	5.9	190,000,000	5.6	5.6	254,000,000	6.0	5.9	280,000,000
6.4	6.4	250,000,000	6.4	6.4	350,000,000	6.4	6.4	940,000,000	6.4	6.4	550,000,000	6.0	6.0	526,000,000	6.2	6.0	270,000,000
6.6	6.6	310,000,000	6.6	6.6	465,000,000	6.6	6.6	1,120,000,000	6.6	6.6	570,000,000	6.4	6.4	690,000,000	6.5	6.6	449,000,000
6.8	6.8	460,000,000	6.8	6.8	480,000,000	6.8	6.8	1,010,000,000	6.8	6.8	530,000,000	6.6	6.6	726,000,000	6.8	6.8	410,000,000
7.0	7.0	430,000,000	7.0	7.0	405,000,000	7.0	7.0	960,000,000	7.0	7.0	540,000,000	6.8	6.8	886,000,000	7.0	7.0	392,000,000
7.2	7.2	410,000,000	7.2	7.2	445,000,000	7.2	7.2	1,010,000,000	7.2	7.2	510,000,000	7.2	7.2	856,000,000	7.6	7.4	456,000,000
7.7	7.7	350,000,000	7.4	7.4	440,000,000	7.6	7.6	710,000,000	7.6	7.6	370,000,000	7.4	7.4	826,000,000	8.0	7.9	386,000,000
8.6	8.1	260,000,000	7.6	7.6	425,000,000							7.8	7.8	640,000,000	8.6	8.5	239,000,000

\* Eldridge isolated from blood of a moderately severe case of typhoid, February, 1919.  
† Eldridge isolated from stool of same patient. Optimum H-ion concentration determined immediately after isolation.  
‡ Isolated from a case of influenza followed by a moderately severe case of typhoid. B. typhosus isolated from sputum.  
§ Isolated from blood in a moderately severe case of typhoid, February, 1919.  
¶ Isolated from blood in a mild case of typhoid in a vaccinated person, February, 1919.

TABLE 7  
EFFECT OF VARYING H-ION CONCENTRATIONS ON GROWTH OF PARATYPHOSUS A AND B

Para A 11* Original Culture			Para A 11† Bile Strain			Para A 11‡ Duodenal Strain			Para A 8§			Para B 3¶		
P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c	P <sub>H</sub> Cont.	P <sub>H</sub> 24-Hour Cul-ture	Growth per C c
4.3	4.3	100	4.3	4.8	33,500	4.3	4.3	300	6.0	6.0	703,000,000	6.0	6.2	740,000,000
4.8	4.8	2,000	5.2	5.1	28,200,000	4.8	4.8	2,500,000	6.4	6.4	746,000,000	6.4	6.4	910,000,000
5.0	5.0	16,300,000	6.4	6.4	636,000,000	5.2	5.1	11,000,000	6.6	6.6	801,000,000	6.6	6.6	960,000,000
6.6	6.6	460,000,000	6.6	6.5	1,003,000,000	6.4	6.4	425,000,000	6.8	6.8	1,266,000,000	6.8	6.8	1,190,000,000
6.8	6.8	513,000,000	6.8	6.8	553,000,000	6.8	6.6	543,000,000	7.0	7.0	1,136,000,000	7.0	7.0	1,410,000,000
7.0	7.0	685,000,000	7.2	7.1	690,000,000	7.2	7.0	543,000,000	7.2	7.2	796,000,000	7.2	7.2	980,000,000
7.5	7.5	705,000,000	7.5	7.4	626,000,000	7.5	7.4	556,000,000	7.4	7.4	896,000,000	7.6	7.6	990,000,000
7.8	8.2	556,000,000	8.6	8.2	396,000,000	8.4	8.0	216,000,000	7.8	7.8	393,000,000	7.8	7.8	830,000,000
8.3	8.6	450,000,000	8.8	8.6	453,000,000	8.8	8.6	184,000,000						
8.8	8.8	105,600,000												
8.9	8.9	>50,000,000#												

\* Received from Captain Nichols. Isolated from Private Fraser in Colorna, Dublin, Mexico, August, 1916.  
† Same strain taken from gallbladder carrier rabbit 816 days after infection.  
‡ Same strain taken from duodenum of same rabbit.  
§ Received from Captain Nichols, March, 1916, isolated from a blood culture.  
¶ Received from the department of bacteriology of the University of Bern, December, 1913.  
# Colonies too numerous to count.

It is evident that the rise in the curve on the acid side is greater than on the alkaline side. In other words, even though *B. typhosus* grows best in a slightly acid environment, it may be slightly more tolerant to alkalis than to acids.

One interesting point brought out by these experiments was that slight changes, near the limiting H-ion concentration, produced more marked changes in growth than approximately the same changes near

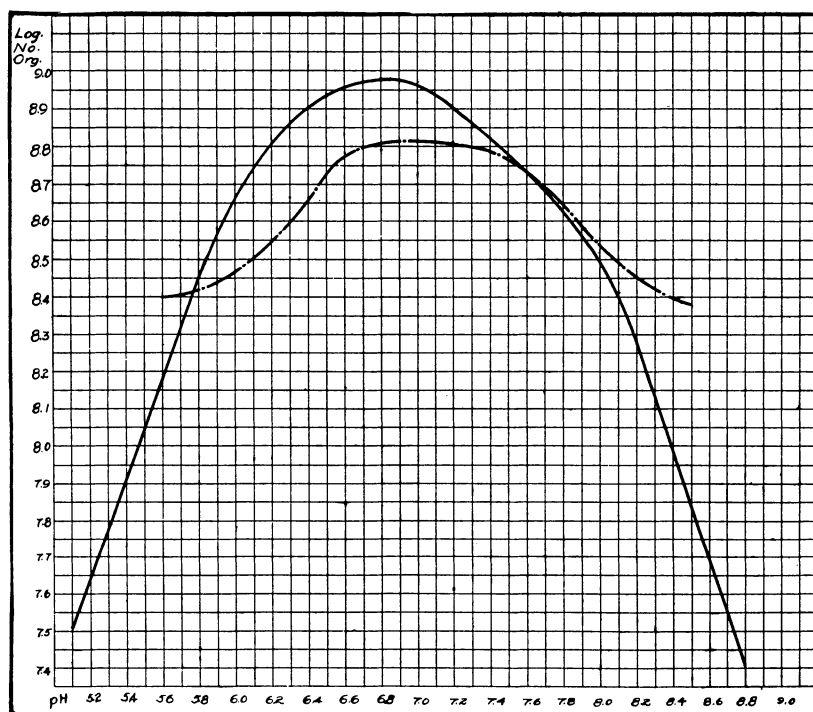


Chart 1.—Growth curve of *B. typhosus*. The growth of stock strains is represented by the unbroken line; the growth of recently isolated strains, by the dash and dot line.

the optimum H-ion concentration. For instance, in the “Blair” strain (table 4), a change from  $P_H + 4.8$ -5.0 caused a change in growth from 97,000 organisms per c c to 41,000,000 organisms per c c; while a change from  $P_H + 6.4$ - $P_H + 6.6$  resulted in a change of 10,000,000 organisms from 480,000,000-580,000,000. The “Kearney” strain failed to grow at  $P_H + 4.3$  and at 4.8, while at  $P_H + 5.2$ , 92,500,000 organisms were

counted per c.c. A change from 6.4 to 6.8 produced no recordable change in growth.

Another important observation was made. Recently isolated cultures had a decided optimal zone of growth which was more marked than in the stock cultures. The dash and dot line in the chart represents the results obtained by averaging 6 experiments conducted with recently isolated strains. Stock cultures exhibited an optimum zone with a definite optimum point of growth, while recently isolated strains developed in an optimum zone. Moreover, the range of growth of the latter strains was greater and the tolerance for alkali was more marked. This was particularly striking with the "C" strain, table 6. The growth of this strain was as profuse at  $P_H + 7.9$  as at  $P_H + 7.0$ . The inhibition at  $P_H + 8.6$  was not as marked as in the stock cultures. These observations were suggestive of bacterial adaptation to alkali and acid, and were considered of utmost importance in connection with the problem of urinary and gallbladder carriers.

These findings have some bearing on the selection of the medium to be employed in the isolation of *B. typhosus*. The optimum reaction for Endo-medium is  $P_H + 7.8$ - $P_H + 8.4$ . Such a concentration of alkali is inhibitive for *B. typhosus*. The optimum reaction for a brilliant green medium is  $P_H + 7.0$ . As far as reaction is concerned, the latter medium is therefore preferable to Endo's agar. It was pointed out by Stickel and Meyer<sup>1</sup> that a negative finding of a stool containing few typhoid bacilli may be misleading when the usual Endo-medium is used. The results obtained in the present study fully confirm these views.

Table 7 shows the effect of varying H-ion concentrations on the growth of paratyphosus A and paratyphosus B.

It is obvious from table 7 that the paratyphoid strains showed an optimum growth at the same H-ion concentration as the typhoid strains, but exhibited a marked plateau on either side of the optimum. The para B strains investigated are more alkali-tolerant than the para A strains.

Para A 2 was isolated from a rabbit which was sacrificed on the 816th day as a chronic gallbladder carrier. The bile and the duodenal strain, being recently isolated, were expected to grow in a plateau curve, but in our tests the reverse was noted, namely, the bile strain showed a decided optimum at  $P_H + 6.5$  while the duodenal strain grew in a

<sup>11</sup> Jour. Infect. Dis., 1918, 23, p. 48.

plateau from  $P_H + 6.6$  to  $P_H + 7.4$ . The original stock culture behaved in a similar manner, namely, a plateau from  $P_H + 6.6$ - $P_H + 7.5$ . In fact, the growth at  $P_H + 7.5$  was slightly greater than at  $P_H + 6.8$ . These differences, which were determined by repeated tests, cannot be explained. It is not unlikely that the prolonged sojourn in a blocked gallbladder, the exposure to various products of inflammation, produced a tolerance to acid. This observation supports the conception that bacteria may undergo a process of adaptation while resident in the tissues of an immune host. Changes in the growth curve may be one of the newly acquired properties of the protoplasm which can be readily recorded. Growth curve studies may therefore be of value in the study of bacterial adaptation.

#### SUMMARY AND CONCLUSIONS

*B. typhosus* has a range of growth from  $P_H + 5.0$  to  $P_H + 8.6$  with an optimum growth at  $P_H + 6.8$ - $P_H + 7.0$  in a salt-free veal infusion broth. Above or below these limits the resulting growth in comparison is very slight.

Large variations in the H-ion concentration near the optimum zone produce only slight effects on the growth of the organisms, while slight variations at the limiting zone produce a marked effect. These observations are fully in accord with the results reported by Cohen and Clark<sup>12</sup> in their studies on the growth of certain intestinal organisms at different concentrations. In the region near the optimum H-ion concentration the tolerance for alkali seems to be slightly greater than for acid.

Stock cultures isolated from stools, blood and urine of typhoid patients or carriers have a more decided optimum than recently isolated cultures of similar cases. In such cultures the plateau of the growth curve is much more pronounced and extends over a wider range than in stock cultures. The latter is suggestive of microbic adaptation to changes in H-ion concentration in body fluids, particularly urine and bile.

*B. paratyphosus* A and B have a range of growth at varying H-ion concentrations similar to that of *B. typhosus* but exhibit a greater tolerance for alkali than *B. typhosus*.

<sup>12</sup> Jour. Bacteriol., 1919, 4, p. 409.